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39/102

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[Continued on next page]

(54) Title: ACTINOBACILLUS PLEUROPNEUMONIAE VIRULENCE GENES

Class	Strain	Gene	Biotherapy Data		Accession	Name of Putative Product	In vitro IC <sub>50</sub>	In vivo IC <sub>50</sub>	
			%	Spores					
Cell Surface	823	cpnC			AAC29191	capsule polysaccharide repeat base subunit	1.639	2.389-02	
	28A-0	tdsB (p4)	2702	62	AAF2181	LPS or capsule biogenesis	1.688		
	987	gpaJ			AAC29192	LPS or capsule biogenesis	1.739		
	1287	tdsJ			AAG4894	LPS O-acetylase biogenesis	0.204		
	10011	tdsK	tdsC (p4)	100	15	AAG4895	LPS O-acetylase biogenesis	0.233	
	1326	tdsH			AAG4892	LPS O-acetylase biogenesis	0.234		
	2188	tdsP			AAG4893	LPS O-acetylase biogenesis	0.623		
	1548	Regulated glycoprotein	PPC708 (P)	2702	276	g118971176	LPS O-acetylase biogenesis	0.739	
	404	gpaB (p4)	4902	89	AAK2999	Staphylococci P <sub>2</sub> promoter	1.079		
	1744	unsp2 (p4)	4002	237	Q4221	Outer membrane protein P2 precursor	1.149		
Membrane	10012	tdsP			P1708	ADP-ribose phosphorylase	1.629		
	10A11	unsp (p4)	6002	34	AAC2373	arabinoside synthase	0.622		
	1307	tdsA (p4)	4902	134	P43714	ATP synthase	0.214		
	1788	unsp1 (p4)	1307	188	P46188	glycerol C <sub>1</sub> dehydrogenase	1.189		
	20011	tdsH (p4)	1307	182	P31318	isomaltose 1-6-glucosyl transferase	1.089	2.439-04	
	20012	tdsH (p4)	6102	394	CA71482	Glutamate decarboxylase	0.249		
	159	tdsH (p4)	1307	114	P45211	polyketide synthase	1.189		
	1806	tdsP			AAC29199	ATPase	0.229		
	22911	unsp (p4)	2902	77	AAC3391	polyketide synthase	1.289	1.049	
	985	gpaB (p4)	7078	123	AAK2992	polyketide synthase	1.189		
Cytoplasm	947	unsp (p4)	429	429	P45715	MDP2 biogenesis	0.719		
	1329	unsp1 (p4)			AAC29201	hydroxyphenyl NADH-ubiquinone reductase	0.719		
	28A18	gpaC (p4)	7301	343	P45716	pyruvate decarboxylase	0.219		
	22912	unsp (p4)	9127	71	P45717	fluorocitrate protein	0.219		
	28A19	tdsC (p4)	4902	188	P45718	Threonine synthase	0.287		
	Q2C	tdsH (p4)	8402	258	Q45119	Swamp synthase	0.249	2.239-02	
	27A12	tdsH			P17916	Swamp synthase	0.192	1.049	
	2806	unsp1 (p4)	8802	258	AAK2919	unsp1 (p4)	0.219		
	13A11	tdsC (p4)	2902	43	AAC29193	unsp1 (p4)	0.284		
	28A8	gpaC (p4)	7078	127	P44585	unsp1 (p4)	0.689		
Regulatory	2387	gpaJ (p4)	7702	157	P44583	polyketide RNA polymerase	0.744		
	28012	tdsH (p4)	1302	43	P44582	GDP synthase	1.884		
	10012	gpaB (p4)	7902	139	PT723	beta galactosyl transferase-2 production	0.227	2.749-02	
	2803	tdsA (p4)	8802	113	P44582	unsp1 (p4)	0.262		
	1806	tdsH (p4)	4288	18	AAC29272	unsp1 (p4)	1.729	0.809-02	
	2129	tdsH (p4)	9102	189	P44702	Swamp synthase	0.719		
	27A12	tdsH (p4)	9102	189	P44702	Swamp synthase	0.719		
	27A12	tdsH (p4)	9102	189	P44702	Swamp synthase	0.719		
	27A12	tdsH (p4)	9102	189	P44702	Swamp synthase	0.719		
	27A12	tdsH (p4)	9102	189	P44702	Swamp synthase	0.719		



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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

# INTERNATIONAL SEARCH REPORT

International Application No  
PCT/GB 03/05349

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N15/31 C07K14/285 C07K16/12 C12N5/10 G01N33/50  
A61K39/102

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12N C07K G01N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the International search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, Sequence Search

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>WO 00/61724 A (PHARMACIA &amp; UPJOHN, INC.) 19 October 2000 (2000-10-19) cited in the application the whole document see especially: SEQ ID NOS: 140, 141 ORF ID: exbB mutant ID: AP11E7 page 42 - page 53; examples 7-11; tables 2-4 and page 233 - page 234</p> <p>----- -/--</p>	<p>1-13, 16-35</p>

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

### \* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "&" document member of the same patent family

Date of the actual completion of the international search

21 June 2004

Date of mailing of the international search report

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## INTERNATIONAL SEARCH REPORT

Internal Application No  
PL 1/GB 03/05349

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 02/075507 A (PHARMACIA & UPJOHN COMPANY) 26 September 2002 (2002-09-26) the whole document see especially: SEQ ID NO: 140, 141 ORF ID: exbB mutant ID: AP11E7 page 45 - page 57; examples 7-11; tables 2-4 and page 234 - page 236	1-13, 16-35
X	----- ELKINS, C. ET AL.: "Role of the Haemophilus ducreyi Ton System in Internalization of Heme from Hemoglobin" INFECTION AND IMMUNITY, vol. 66, no. 1, January 1998 (1998-01), pages 151-160, XP002285338	16-27, 30-33
A	the whole document  see especially: page 154 - page 155; figure 2 ExbB, ExbD, TonB proteins and page 158, column 2, line 26 - line 37	1-13,28, 29,34,35
A	----- FULLER, T.E. ET AL.: "A genetically-defined riboflavin auxotroph of Actinobacillus pleuropneumoniae as a live attenuated vaccine" VACCINE, vol. 18, no. 25, 15 June 2000 (2000-06-15), pages 2867-2877, XP004203577 the whole document	1-13, 16-35
T	----- BEDDEK, A.J. ET AL.: "Two TonB Systems in Actinobacillus pleuropneumoniae: Their Roles in Iron Acquisition and Virulence" INFECTION AND IMMUNITY, vol. 72, no. 2, February 2004 (2004-02), pages 701-708, XP008031967 the whole document -----	1-13, 16-35

# INTERNATIONAL SEARCH REPORT

tional application No.  
PCT/GB 03/05349

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
  
2. ☒ Claims Nos.: 14, 15, 36-40 (completely) and 17, 18, 29-33 (partially)  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:  
see FURTHER INFORMATION sheet PCT/ISA/210
  
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
  
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
  
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
  
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:  
1-13, 16-35 (partially)

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

## FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 14, 15, 36-40 (completely) and 17, 18, 29-33 (partially)

Present claims 14 and 15 and dependent claims 17, 18, 29-33 relate to a polynucleotide defined by reference to a desirable characteristic or property, namely, for claim 14: "encoding a gene product which is not naturally found in *A. pleuropneumoniae*, but whose expression therein is capable of modulating ... the virulence of that bacterium", and for claim 15: "which is not naturally found in *A. pleuropneumoniae* but which is capable of modulating the virulence of that bacterium by its direct interaction with *A. pleuropneumoniae* virulence genes or gene products".

The claims cover all polynucleotides having this characteristic or property, whereas the application provides no support within the meaning of Article 6 PCT and no disclosure within the meaning of Article 5 PCT for such polynucleotides. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search is impossible. Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define the polynucleotide by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search impossible. Consequently, no search has been carried out for claims 14 and 15 and for dependent claims 17, 18, 29-33.

Furthermore, present claims 36, 38-40 relate to an anti-bacterial agent defined by reference to a desirable characteristic or property, namely "identified by the method of claims 34 or 35".

The claims cover all compounds having this characteristic or property, whereas the application provides no support within the meaning of Article 6 PCT and no disclosure within the meaning of Article 5 PCT for such compounds. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search is impossible. Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define the compound by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search impossible. Consequently, no search has been carried out for claims 36, 38-40.

The same applies to claim 37 relating to a "method of modulating the transcription of such virulence genes through the use of oligonucleotide-directed triplet helix formation". However, the application provides no support within the meaning of Article 6 PCT and no disclosure within the meaning of Article 5 PCT for such oligonucleotides. Accordingly, no search has been carried out for claim 37.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international

## FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

search report has been established need not to be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

The applicant's attention is drawn to the fact that claims relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure. If the application proceeds into the regional phase before the EPO, the applicant is reminded that a search may be carried out during examination before the EPO (see EPO Guideline C-VI, 8.5), should the problems which led to the Article 17(2) declaration be overcome.

## FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-13, 16-35 (partially)

An attenuated *Actinobacillus pleuropneumoniae* bacterium having a mutation in a gene required for bacterial virulence which comprises the nucleotide sequence of SEQ ID NO: 1, a composition containing said attenuated *A. pleuropneumoniae* bacterium, use of said attenuated *A. pleuropneumoniae* bacterium in the manufacture of a medicament for preventing or alleviating an infection of an animal with *A. pleuropneumoniae*, an isolated polynucleotide comprising a) a nucleotide sequence of SEQ ID NO: 1, b) a nucleotide sequence encoding the polypeptide which is encoded by the nucleotide sequence recited in a), c) a nucleotide sequence which hybridizes to the nucleotide sequence of a) and/or b) or to its complement under conditions of moderate to high stringency, d) a fragment of any one of the nucleotide sequences of a)-c) which fragment retains an immunological properties and/or biological activity of the recited nucleotide sequence, a vector comprising said polynucleotide, a host cell comprising said vector, an isolated *A. pleuropneumoniae* polypeptide encoded by said polynucleotide, a method of producing said polypeptide, a composition containing said polypeptide, an antibody which specifically recognizes said polynucleotide or said polypeptide, a method for identifying an anti-bacterial agent which is capable of modulating the function of the said *A. pleuropneumoniae* virulence gene, an attenuated bacterium containing a mutation in a gene comprising a nucleotide sequence which is capable of hybridising to the nucleotide sequence defined by SEQ ID NO: 1 under consitions of moderate to high stringency, a composition containing said attenuated bacterium, use of said attenuated bacterium in the manufacture of a medicament for the therapeutic treatment or prophylactic protection of an animal against infection by the corresponding wild-type bacterium, an isolated virulence polypeptide encoded by said gene, a composition containing said polypeptide, an antibody which specifically recognizes said polynucleotide or said polypeptide;

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2. claims: 1-13,16-35 (partially)

idem as subject 1, but limited to SEQ ID NO: 2;

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# INTERNATIONAL SEARCH REPORT

International Application No  
PCT/GB 03/05349

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 0061724	A	19-10-2000	AU 4077600 A BR 0009663 A CA 2366520 A1 CN 1351653 T EP 1171577 A2 JP 2002541790 T NZ 514883 A WO 0061724 A2 US 2004110268 A1 ZA 200108262 A	14-11-2000 09-04-2002 19-10-2000 29-05-2002 16-01-2002 10-12-2002 26-03-2004 19-10-2000 10-06-2004 08-01-2003
WO 02075507	A	26-09-2002	US 2004110268 A1 CA 2438315 A1 EP 1368456 A2 WO 02075507 A2	10-06-2004 26-09-2002 10-12-2003 26-09-2002